4-2021

Niche differentiation of bacteria and fungi in carbon and nitrogen cycling of different habitats in a temperate coniferous forest: A metaproteomic approach

Robert Starke
Institute of Microbiology of the Czech Academy of Sciences

Rubén López Mondéjar
Institute of Microbiology of the Czech Academy of Sciences

Zander Rainer Human
Institute of Microbiology of the Czech Academy of Sciences

Diana Navrátilová
Institute of Microbiology of the Czech Academy of Sciences

Martina Štursová
Institute of Microbiology of the Czech Academy of Sciences

See next page for additional authors

Niche differentiation of bacteria and fungi in carbon and nitrogen cycling of different habitats in a temperate coniferous forest: A metaproteomic approach

Abstract

Temperate coniferous forests sustain the highest levels of biomass of all terrestrial ecosystems and belong to the major carbon sinks on Earth. However, the community composition and its functional diversity depending on the habitat have yet to be unveiled. Here, we analyzed the proteomes from litter, plant roots, rhizosphere, and bulk soil in a temperate coniferous forest at two time points to improve the understanding of the interplay between bacterial and eukaryotic communities in different habitats. Our metaproteomic approach yielded a total of 139,127 proteins that allowed to differentiate the contribution of microbial taxa to protein expression as well as the general functionality based on KEGG Orthology in each habitat. The pool of expressed carbohydrate-active enzymes (CAZymes) was dominated by fungal proteins. While CAZymes in roots and litter targeted mostly the structural biopolymers of plant origin such as lignin and cellulose, the majority of CAZymes in bulk and rhizosphere soil targeted oligosaccharides, starch, and glycogen. Proteins involved in nitrogen cycling were mainly of bacterial origin. Most nitrogen cycling proteins in litter and roots participated in ammonium assimilation while those performing nitrification were the most abundant in bulk and rhizosphere soil. Together, our results indicated niche differentiation of the microbial involvement in carbon and nitrogen cycling in a temperate coniferous forest topsoil.

Keywords

Temperate coniferous forest, Metaproteome, Habitats of topsoil, Carbon and nitrogen, Cycling, CAZymes

Disciplines

Bioresource and Agricultural Engineering | Environmental Microbiology and Microbial Ecology | Forest Biology

Comments

This article is published as Robert Starke, Rubén López Mondéjar, Zander Rainer Human, Diana Navrátilová, Martina Štursová, Tomáš Větrovský, Heather M. Olson, Daniel J. Orton, Stephen J. Callister, Mary S. Lipton, Adina Howe, Lee Ann McCue, Christa Pennacchio, Igor Grigoriev, and Petr Baldrian. "Niche differentiation of bacteria and fungi in carbon and nitrogen cycling of different habitats in a temperate coniferous forest: A metaproteomic approach." *Soil Biology and Biochemistry* (2021): 108170. DOI: 10.1016/j.soilbio.2021.108170.

Authors

Robert Starke, Rubén López Mondéjar, Zander Rainer Human, Diana Navrátilová, Martina Štursová, Tomáš Větrovský, Heather M. Olson, Daniel J. Orton, Stephen J. Callister, Mary S. Lipton, Adina Howe, Lee Ann McCue, Christa Pennacchio, Igor Grigoriev, and Petr Baldrian

Soil Biology and Biochemistry 155 (2021) 108170

Contents lists available at ScienceDirect

Soil Biology and Biochemistry

journal homepage: http://www.elsevier.com/locate/soilbio

Niche differentiation of bacteria and fungi in carbon and nitrogen cycling of different habitats in a temperate coniferous forest: A metaproteomic approach

Robert Starke [a,*], Rubén López Mondéjar [a], Zander Rainer Human [a], Diana Navrátilová [a], Martina Šťursová [a], Tomáš Větrovský [a], Heather M. Olson [c], Daniel J. Orton [b], Stephen J. Callister [b], Mary S. Lipton [c], Adina Howe [d], Lee Ann McCue [c], Christa Pennacchio [e], Igor Grigoriev [e], Petr Baldrian [a]

[a] Laboratory of Environmental Microbiology, Institute of Microbiology of the Czech Academy of Sciences, Vídeňská 1083, 14220, Praha 4, Czech Republic
[b] Biological Science Division, Pacific Northwest National Laboratory, Richland, WA, 99352, USA
[c] Environmental Molecular Sciences Laboratory, Pacific Northwest National Laboratory, Richland, WA, 99352, USA
[d] Department of Agricultural and Biosystems Engineering, Iowa State University, Ames, IA, 50011, USA
[e] Department of Energy Joint Genome Institute, 2800 Mitchell Drive, Walnut Creek, CA, 94598, USA

ARTICLE INFO

Keywords:
Temperate coniferous forest
Metaproteome
Habitats of topsoil
Carbon and nitrogen
Cycling
CAZymes

ABSTRACT

Temperate coniferous forests sustain the highest levels of biomass of all terrestrial ecosystems and belong to the major carbon sinks on Earth. However, the community composition and its functional diversity depending on the habitat have yet to be unveiled. Here, we analyzed the proteomes from litter, plant roots, rhizosphere, and bulk soil in a temperate coniferous forest at two time points to improve the understanding of the interplay between bacterial and eukaryotic communities in different habitats. Our metaproteomic approach yielded a total of 139,127 proteins that allowed to differentiate the contribution of microbial taxa to protein expression as well as the general functionality based on KEGG Orthology in each habitat. The pool of expressed carbohydrate-active enzymes (CAZymes) was dominated by fungal proteins. While CAZymes in roots and litter targeted mostly the structural biopolymers of plant origin such as lignin and cellulose, the majority of CAZymes in bulk and rhizosphere soil targeted oligosaccharides, starch, and glycogen. Proteins involved in nitrogen cycling were mainly of bacterial origin. Most nitrogen cycling proteins in litter and roots participated in ammonium assimilation while those performing nitrification were the most abundant in bulk and rhizosphere soil. Together, our results indicated niche differentiation of the microbial involvement in carbon and nitrogen cycling in a temperate coniferous forest topsoil.

1. Introduction

A large share of the Earth's carbon (C) resides in living trees, forest vegetation and soil microbial biomass of temperate coniferous forests (Pan et al., 2011). Understanding their functional responses to global change is important for predictive climate modelling as they act as both C sources and sinks. The C cycling in forest topsoil is highly integrated within the biosphere. Its complex dynamics are influenced by both soil community functions and a heterogeneous trophic environment. Soil microbes contribute to a variety of ecosystem functions such as the decomposition of organic matter, tree root symbiosis and pathogenicity. Microbes inhabiting litter, on the other hand, depend on complex litter-bound nutrients recycled by microbial decomposition that is partly mineralized and partly transformed into organic matter, which accumulates in the forest floor (Baldrian, 2017). Plant root symbionts, especially mycorrhizal fungi, act as important regulators of plant productivity and are responsible for the acquisition of limiting nutrients such as nitrogen (N) or phosphorus (Van Der Heijden et al., 2008), especially in nutrient-poor ecosystems (Franklin et al., 2014). Mycorrhizal fungi dominate the fungal community in the coniferous forest soils below the litter layer (Lindahl et al., 2007) and represent potentially the largest pool of microbial biomass (Ekblad et al., 2013). By

* Corresponding author.
E-mail address: robert.starke@biomed.cas.cz (R. Starke).

https://doi.org/10.1016/j.soilbio.2021.108170
Received 27 May 2020; Received in revised form 3 February 2021; Accepted 5 February 2021
Available online 9 February 2021

extending the tree roots by their hyphae, they mediate the flow of tree-derived C through its roots into the soil, which can account for more than 30% of total primary production in these ecosystems (Clemmensen et al., 2013; Franklin et al., 2014). The topsoil clearly represents the major compartment of C storage and microbial activity in coniferous forests but understanding the processes of C and nutrient cycling is, however, complicated due to its spatial heterogeneity resulting from the existence of four habitats: litter, plant roots, rhizosphere, and bulk soil that are distinct in many respects (Baldrian, 2017). In addition to the fact that soil and rhizosphere contains smaller amount of organic matter than litter and roots, roots represent a habitat where the bulk of the plant biomass is living and thus not readily degraded. Furthermore, spruce litter and roots also differ in the content of major biopolymers: brown spruce needles contain around 32% lignin, 29% cellulose and 21% hemicelluloses (Johansson, 1995) while fine spruce roots (<2 mm) are reported to contain more lignin (34%) and less cellulose (20%) and hemicelluloses (9% (Hobbie et al., 2010);). Therefore, the application of the same analytical tools to all these habitats is the best option to understand and to relate their functions in ecosystem processes that reflect their properties.

Specific functions of the topsoil organisms can be statistically inferred based upon the homology of genes, transcripts or proteins that are present to experimentally characterized genes and proteins in known organisms. This so-called ortholog annotation is performed by the comparison to phylogenomic databases, covering either a wide range of functional classes, e.g., KEGG Orthology (Kanehisa et al., 2016a,b) or more specific subsets, such as the carbohydrate-active enzymes (CAZymes) (Lombard et al., 2014) involved in C cycling. Processes of C cycling are closely related to N cycling as their ratio can be used to identify the origin of a source (Müller, 1977; Ishiwatari and Uzaki, 1987; Prahl et al., 1994). The theory of ecological stoichiometry suggests that strictly homeostatic organisms have low nitrogen use efficiency (NUE) but high carbon use efficiency (CUE) at low substrate C/N-ratios (Sterner and Elser, 2002). By contrast, these organisms are expected to lower their CUE while increasing their NUE at high substrate C/N-ratios (Mooshammer et al., 2014). Logically, there exists a threshold elemental ratio (TER) that defines the elemental ratio at which the metabolic control of an ecological system switches from C limitation to N limitation (Urabe and Watanabe, 1992; Anderson and Hessen, 1995). The result is an inverse relationship between C and N cycling separated by the TER (Mooshammer et al., 2014) that may yield a different set of partaking microbes (Starke, 2017). In coniferous forest soils, a variety of C sources of different complexity are decomposed with a vast arsenal of glycoside hydrolases (GHs) that hydrolyze glycosidic bonds (Lombard et al., 2014). The functional classification comprises families of structurally-related catalytic and carbohydrate-binding modules or functional domains of enzymes that degrade, modify or create glycosidic bonds (Lombard et al., 2014), and are thus important for the breakdown of the different components of biomass: cellulose, hemicellulose and pectin from plant, chitin from fungal and peptidoglycan from bacterial cell walls. Importantly, however, members of the same CAZymes family can catalyze different reactions and their family membership may not sufficiently indicate the targets of their activity (López-Mondéjar et al., 2016) as seen in the family GH5. In addition to GHs, CAZymes include glycosyl transferases (GTs), carbohydrate esterases (CEs) and polysaccharide lyases (PLs) involved in the decomposition processes as well as auxiliary activities (AA) that cover redox enzymes acting in conjunction with CAZymes. The AA class is key for the decomposition of lignin by oxidative enzymes (Levasseur et al., 2013). On the other hand, N is available in the environment in different chemical forms: organic nitrogen, ammonium, nitrite, nitrate, nitrous oxide, nitric oxide, and inorganic nitrogen gas. Several enzymes predominantly produced by microorganisms are involved in their interconversions during assimilation, denitrification, nitrification or nitrogen fixation (Simon and Klotz, 2013; Sparacino-Watkins et al., 2014). For the global N budget, nitrogen fixation in natural ecosystems (Vitousek et al., 2013) as well as agricultural systems (Fowler et al., 2013), the emission of oxidized N forms from soil (Pilegaard, 2013) and of ammonium in terrestrial ecosystems (Sutton et al., 2013) were previously deemed important.

The use of metaproteomics became more and more popular to study how microbes contribute to soil ecosystem services (Von Bergen et al., 2013) as proteins not only provide both functional and taxonomic information (Hettich et al., 2013; Von Bergen et al., 2013; Wilmes et al., 2015), but also represent the catalysts of important biochemical reactions (Wong, 2009). In favor of using proteins are: (i) the extracellular activity of soil proteins that can persist and remain active through the stabilization by humic substances and clay (Burns et al., 2013) whereas up to 40% of DNA (Carini et al., 2016) and 6% of RNA (Papp et al., 2018) were reported to derive from dead cells; (ii) the limitations of nucleic acids sequencing such as quantitative accuracy (Feinstein et al., 2009; Hungate et al., 2015), which may result in biased proportions of community members or functions; (iii) the difference between mRNA and protein levels depending on the temporal scale, on the complexity of the biological system and on the type of perturbation (Liu and Aebersold, 2016). Methodological advances, such as two-dimensional liquid chromatography (Callister et al., 2018) and site specific databases from sequencing approaches, now allow for a high protein identification rate that makes it possible to detect proteins with low relative abundance, such as CAZymes or proteins related to N cycling. Even though they are essential for the ecosystem functioning, they make up only a small part of the proteome that is dominated by structural proteins and proteins participating in the central metabolism.

In this study, we used a metaproteomic approach combining a site specific database comprising metagenomes, metatranscriptomes and fungal genomes together with two-dimensional liquid chromatography (Callister et al., 2018) to unveil the taxonomic and functional composition of the metaproteome in a temperate coniferous forest. In addition to describing the general functionality using KEGG Orthology, we have specifically addressed CAZymes that are involved in C cycling and proteins involved in N cycling. Four contrasting habitats – litter, plant roots, rhizosphere, and bulk soil, were compared to unveil habitat specific functions. We hypothesized that the utilization of plant biopolymers performed by fungi will be highest in litter, while components of microbial biomass targeted largely by bacteria (Lladó et al., 2017; López-Mondéjar et al., 2018) will be more important in soil and rhizosphere. Due to the high C/N-ratio and the high share of biomass-derived C in litter, we also expected that the incorporation of ammonia will be higher in litter compared to rhizosphere and soil where the share of inorganic N is higher and the C/N-ratio is lower (Baldrian et al., 2012).

2. Material and methods

2.1. Study area and sample collection

The study area was located at high altitudes (1170–1200 m) of the Bohemian Forest mountain range (Central Europe; 49°02 N, 13°37 E) and was covered by an unmanaged Norway spruce (*Picea abies* (L.) H. Karst.) forest. The mean annual temperature was 5 °C and the mean annual precipitation was 1000 mm. The understory was either missing or composed of grasses (*Avenella*, *Calamagrostis*), bilberries (*Vaccinium*) and mosses. The same study area was explored previously to identify the total and active microbial communities and the analysis of microbiome activity (Baldrian et al., 2012; Žifčáková et al., 2016). To account for potential seasonal variation, samples were collected at two time points, on 09/22/2015 - peak of vegetation season represented by the highest belowground allocation of photosynthates (Hansson et al., 2013) and on 03/22/2016 (end of winter). At five sites, located approximately 250 m from each other, sixteen soil cores (4 cm diameter) were collected from a defined area of 3 × 3 m. Litter (2–4 cm thickness), soil (organic horizon, 3–6 cm of depth), rhizosphere soil (soil adhering to plant roots that had to be manually detached) and plant roots (<2 mm in diameter) themselves were separated from each other. Organic horizon of soil was

selected for sampling since it contains the bulk of the microbial biomass, roots, and ectomycorrhizal mycelia in this system (Žifčáková et al., 2016). Moreover, due to the stony nature of the site, mineral horizon is often present only as random pockets between stones and cannot be reasonably sampled. Litter was cut into 0.5 cm pieces and mixed, soil was passed through a 5-mm sterile mesh and mixed, and the same was later done for rhizosphere soil. Due to the need for immediate freezing in liquid nitrogen, roots were washed on site in sterile deionized water until all visible soil material was removed. All root material was pooled per site and sampling time resulting in a total of 40 samples (5 sites × four habitats × two seasons). All samples were immediately frozen in liquid nitrogen and stored on dry ice. Samples for nucleic acid extraction were stored at −80 °C, samples for metaproteome analysis were freeze-dried and stored at −45 °C.

2.2. Litter and soil chemistry

Soil physicochemical parameters were measured in an external laboratory. Litter, soil and root samples were powdered prior to all analyses. Powdered root samples were included in C and N analysis. pH was measured by mixing powdered samples with either 5 mL of deionized water or KCl shaken for 5 min on orbital shaker before measurement on a laboratory pH meter (WTW Multilab 540). C and N content was measured by combustion of powdered material in 100% O2 at 1000 °C, and the amount of oxidized C and N recorded in a Flash 2000 elemental analyser (Thermo Scientific) and analyzed with Eager Xperience software (Thermo Scientific) to get its relative content. Organic carbon and carbonate content were measured with a second, parallel analysis of the sample after decomposition with hydrochloric acid and fumigation of the released carbon dioxide. Nitrate and ammonium ions were extracted in 0.5 M potassium sulphate solution, filtered, and then quantified by injection into a Quikchem FIA 8000 (Lachat Instruments, Colorado, USA) autoanalyser instrument and processed using Omnion 3.0 software (Lachat Instruments, Colorado, USA). Available Ca, K, and Mg was measured after extraction in Mehlich II solution in a 1:5 sample: extractant ratio and then quantified by atomic absorption spectrophotometry in a Analytik Jena ConrAA 700 (Jena, Germany).

2.3. Protein extraction

Protein extractions were performed according to previously described protocols (Nakayasu et al., 2016; Callister et al., 2018) with minor modifications. Extractions were performed from 2 g of lyophilized rhizosphere soil and bulk soil samples. Briefly, proteins were extracted by the sequential addition of Millipore ultrapure water (Millipore, Billerica, MA), ice-cold methanol and chloroform to a final ratio of 8:4:2 chloroform:methanol:water (v/v). A mixture of 0.9–2.0 mm stainless steel beads, 0.1 mm garnet beads and 0.1 mm zirconia beads were added to these samples. Cells were lysed using a combination of horizontal vortexing and sonication with a probe sonicator at 20% amplitude for 30 s. Protein extractions from roots and litter were performed from 1 g of lyophilized samples reconstituted in Millipore, ultrapure water. Samples were homogenized with an Omni handheld homogenizer (Omni International, Marietta, GA) with disposable tips after adding ice-cold methanol. After homogenization, chloroform was added to obtain a final ratio of 8:4:2 chloroform:methanol:water (v/v). The aqueous and organic phases of the extracts were separated by centrifugation, and after discarding the upper methanol phase, the middle chloroform interphase and the bottom debris pellets were collected in separate clean 50 mL tubes. The protein interphase and debris pellets were precipitated in methanol, and the resulting pellets resuspended in a protein solubilization buffer (4% SDS and 100 mM DTT in 50 mM Tris buffer, pH 8.0, aided by sonication and shaking. A final concentration of 20% trichloroacetic acid was added to the clean supernatants, after which it was centrifuged, and the pellets collected. These protein pellets were washed three times in acetone, and in the final wash step, the protein-containing solutions were transferred to 2 mL tubes and the pellets collected by centrifugation. The Filter-Aided-Sample-Preparation (FASP, Expedeon LTD, UK) digestion method was used for digestion of protein pellets. Protein pellets were resuspended in UPX buffer (Expedeon LTD, UK) and transferred to the spin columns provided in the FASP kit along with 8 M urea in 100 mM ammonium bicarbonate. Spin columns were washed three times with a urea buffer, followed by two washes with 25 mM ammonium bicarbonate. The spin columns were then transferred to clean collection tubes, and protein digestion performed in 100 mM NH_4HCO_3 and 4 μg trypsin on the spin columns. The digested peptides were collected by centrifugation, and any remaining peptides were collected with another addition of NH_4HCO_3. The eluent was concentrated to 30 μL using a SpeedVac at room temperature and a vacuum of 1 Torr. The concentrations were measured with a bicinchoninic acid (BCA) assay (Thermo Fisher Scientific, Waltham, MA). Samples were diluted to 0.1 μg per μL for LC-MS analysis.

2.4. Mass spectrometric measurement

Peptides from each sample were separated using two different orthogonal separation strategies directly coupled to the mass spectrometer generating 12 fractions and 12 MS/MS datasets per sample (Callister et al., 2018) with a run time of 26 h per sample for a total of 562 MS/MS datasets. For the first and additional dimension of liquid chromatography to traditional mass spectrometric measurements, peptides were diluted in 10 mM NH_4HCO_2 (pH 10.0) and separated into 12 fractions using a commercial Waters (Milford, MA) XBridge 5 μm particle size C18 column (4.6 mm i. d. x 250 mm length) with an attached 20 mm long ×4.6 mm i. d. guard column at 0.5 mL min^{-1} using an Agilent 1100 series HPLC system (Agilent Technologies, SantaClara, CA) with two mobile phases: A) 10 mM NH_4HCO_2 (pH 10.0), and B) 10 mM NH_4HCO_2 (pH 10.0) with acetonitrile (v/v 10:90) and eluted with increasing concentrations of B from 0% to 70% over 120 min. All fractions were dried under vacuum and resuspended in 15 μl 10 mM NH_4HCO_2 (pH 10.0). For the second dimension of liquid chromatography, the peptides of each of the 12 fractions were separated and eluted with the same setup as describe above but, instead of fractionating the eluted peptides, the peptides were ionized with electrospray emitters made in-house using 150 μm o.d. x 20 μm i.d. chemically etched fused silica (Kelly et al., 2006) and sprayed into the tandem mass spectrometry (MS/MS), a Q-Exactive HF mass spectrometer (Thermo Scientific, San Jose, CA) that included a Pacific Northwest National Laboratory (PNNL)-designed nano-electrospray ionization interface (Callister et al., 2018). The ion transfer tube temperature and spray voltage were 300°C and 2.2 kV, respectively. For each fraction, data were collected for 100 min. Mass spectra were acquired from 400 to 2000 m z^{-1} at a resolution of 60 k and automatic gain control (AGC) target of 3×10^6. The top 12 precursors were selected for higher-energy collisional dissociation (HCD) MS/MS spectra acquired in data-dependent mode with an isolation window of 2.0 Th and at a resolution of 17,500 (AGC target of 1×10^5) using a normalized collision energy of 30 and a 30 s dynamic exclusion time.

2.5. Database generation and metaproteome annotation

MSConvert (Mayampurath et al., 2008) was used to convert proteome datasets into.dta search files in preparation for amino acid sequence assignment using the MS-GF + search algorithm (Kim et al., 2008) in combination with the study site specific database comprising metagenomes and metatranscriptomes that derived from the same samples as the proteins. To obtain these, DNA and RNA were extracted and libraries were prepared as described previously (Žifčáková et al., 2016, 2017) except for the spruce roots where RNA was extracted and purified using the Nucleospin RNA Plant Kit (Macherey Nagel). All libraries were sequenced on an Illumina HiSeq2000 in the Joint Genome Institute, Walnut Creek, USA, to generate 150-base paired-end reads.

Metagenome and metatranscriptome samples were assembled as described previously (Žifčáková et al., 2016, 2017). Briefly, Trimmomatic 0.36 (Bolger et al., 2014) and FASTX-Toolkit (http://hannonlab.cshl.edu/fastx_toolkit/) were used to remove adaptor contamination, trim low-quality ends of reads and omit reads with overall low quality (<30), sequences shorter than 50 bp were omitted. Combined assembly of all samples was performed using MEGAHIT 1.1.3 (Li et al., 2015). Despite high sequencing depth of the metagenome, only a few tens of predicted metagenome-assembled genomes (MAGs) were obtained that covered a small share of the total diversity (Nayfach et al., 2020). It was thus impossible to utilize MAGs for annotation or analysis purposes. Gene calling and annotation was performed using JGI Gold as well as using BLAST against all published fungal genomes available in January 2018 (Grigoriev et al., 2014). Of these two, the taxonomic identification with a higher bitscore was used as the best hit. Genes encoding CAZymes were annotated using the dbCAN HMM database V6 (Huang et al., 2018). CAZymes were grouped based on their participation in the utilization of distinct C sources based on their classification to known CAZymes families (Lombard et al., 2014). For genes predicted in the metagenome and metatranscriptome assemblies, taxonomic annotations with a bitscore lower than 135 and functional annotations with e-values higher than $1e^{-30}$ were disregarded. The phyla *Chordata, Streptophyta* and *Coniferophyta* were not analyzed. Functional analysis comprised metabolism, cellular processes, environmental information processing and genetic information processing. Metagenome and metatranscriptome data are available at IMG Integrated Microbial Genomes of the JGI DOE (Taxon object IDs 3300031708 and 3300032515 (Chen et al., 2019)).

Search parameters included a 20-ppm precursor mass tolerance dynamic modification of methionine (oxidation at +15.9949 Da), static modification of cysteine residues resulting from alkylation during sample preparation, and the inclusion of partially tryptic peptides. Protein abundances as the area under the curve of proteins with a false discovery rate (FDR) below 0.05 based on the propensity score matching (PSM) score. In total, 139,127 proteins were identified by our metaproteomic approach. Proteins assigned to *Chordata* were disregarded as those are likely either human origin or wrongly annotated. Proteins assigned to the plants *Coniferophyta* and *Streptophyta* were discarded from the microbiome analysis. The significance of treatment effects on total protein content, the relative abundance of ribosomal proteins, CAZymes, N cycling and general functionality on level 2 and level 3 KEGG Orthology from the different habitats were calculated based on the Tukey's honestly significance difference (HSD) test (Tukey, 1949; Morrison et al., 2013). Numbers of proteins per time and habitat that were measured and annotated by taxonomy and function can be found in Table 1.

Table 1
Chemistry of the litter, roots, and soil (each n = 5).

	Litter	Roots	Soil
pH (H_2O)	3.51 ± 0.05	n.d.	3.36 ± 0.07
pH (KCl)	2.92 ± 0.02	n.d.	2.82 ± 0.06
N (%)	1.82 ± 0.16	1.04 ± 0.04	1.22 ± 0.15
$N–NH_4^+$ (mg kg^{-1})	10.8 ± 4.12	n.d.	9.73 ± 2.68
$N–NO_3^-$ (mg kg^{-1})	5.83 ± 1.17	n.d.	6.70 ± 1.20
C (%)	50.3 ± 3.19	41.23 ± 0.94	29.2 ± 5.42
C_{Org} (%)	47.4 ± 2.94	n.d.	23.6 ± 7.55
$C_{Carbonate}$ (%)	2.91 ± 1.29	n.d.	5.60 ± 2.45
C/N-ratio	27.7 ± 3.18	39.64 ± 0.96	23.9 ± 5.44
Ca (mg kg^{-1})	504 ± 114	n.d.	235 ± 124
Mg (mg kg^{-1})	220 ± 39.7	n.d.	116 ± 30.5
K (mg kg^{-1})	490 ± 138	n.d.	171 ± 32.3

n.d. – not determined due to insufficient mass.

3. Results

3.1. Chemistry of litter, roots, and soil

Litter showed a significantly higher pH (P = 0.0055 with H_2O and 0.0171 with KCl) than soil (Table 1). Total nitrogen was significantly higher in litter as compared to soil (P = 0.0003) where both ammonium and nitrate were comparable between the two habitats. Both total carbon (P = 0.0002) and organic carbon (P = 0.0011) was significantly higher in litter while carbonate carbon was higher in soil (P = 0.0726). Calcium (P = 0.0074), magnesium (P = 0.0020), and potassium (P = 0.0054) were all significantly higher in litter as compared to soil. Nitrogen content in the roots was significantly lower (P < 0.05) as compared to soil and litter. Otherwise, the carbon content in the roots was significantly higher than in soil but significantly lower than in litter (P < 0.05). Together, this resulted in significantly higher C/N-ratios in roots as compared to litter and soil (P < 0.05).

3.2. Protein content and the relative abundance of ribosomal proteins, CAZymes and proteins related to N cycling

Plant roots in September showed significantly (P < 0.05) higher protein counts compared to litter in March and September, which in turn contained significantly more proteins than found in plant roots in March as well as bulk and rhizosphere soil regardless the sampling time (Fig. 1a, Table 2). The relative abundance of ribosomal proteins from plants was significantly higher in spruce roots regardless the sampling time than in litter, bulk, and rhizosphere soil where the share of plant proteins was relatively low (Fig. 1b). In total, the ribosomal proteins from non-plant proteins made up approximately 0.5% in spruce roots, bulk, and rhizosphere soil and up to 2% in litter. The relative abundance of plant proteins assigned to CAZymes was significantly higher in spruce roots sampled in March compared to September and higher than in the other habitats regardless the sampling time. The relative abundance of non-plant CAZymes ranged from 0.2% in roots to 2.0% in rhizosphere soil. Plant proteins related to N cycling were significantly higher in roots regardless the sampling time but showed no significant contribution in litter, bulk, and rhizosphere soil. Non-plant proteins related to N cycling ranged from 0.02% in roots 0.87% in rhizosphere soil.

3.3. Total and ribosomal microbial community composition, and its general functionality

The total protein pool after the exclusion of the plant proteins was dominated by bacteria regardless of habitat and the sampling time with higher proportions of eukaryotes in roots and to a lesser extent in litter (Fig. 2). Particularly *Proteobacteria*, *Actinobacteria* and *Acidobacteria* were the dominant bacterial phyla. Archaeal proteins were most abundant in bulk and rhizosphere soil during March, representing up to 0.2% and 0.1% relative protein abundance, respectively, but only 0.01–0.03% in the other habitats and sampling times. The fungal *Basidiomycota* and *Ascomycota* dominated among the eukaryotes regardless of the habitat or the sampling time, with a higher *Basidiomycota*-to-*Ascomycota* ratio in roots and litter without differences with sampling time. The composition of ribosomal proteins showed a much larger contribution of eukaryotes in roots, reaching approximately 65% of the total and in litter (up to 50%). The ribosomal proteins were dominated by the fungi from the *Basidiomycota* and *Ascomycota* like the total proteins, but higher abundances of *Arthropoda* were found particularly in roots.

In total, 16 of the 19 most abundant level 2 KEGG categories and 22 of the 26 most abundant level 3 categories showed significant differences between habitats in their relative contribution to the total proteome (Fig. 3). Particularly on level 2, proteins involved in amino acid metabolism, cell growth and death, energy metabolism, signal transduction, translation and transport and catabolism were highly abundant in roots. Rhizosphere soil showed high number of proteins involved in

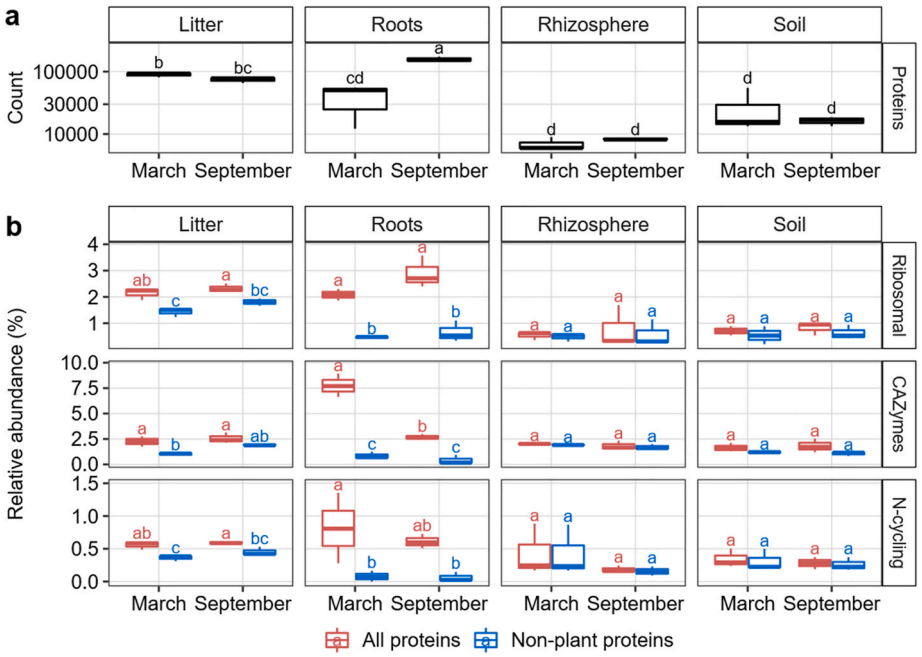

Fig. 1. Count of protein groups identified by at least one unique peptide in logarithmic scale (a). The relative abundance of ribosomal proteins and of proteins affiliated to CAZymes or nitrogen cycling (b). Data with the same letter across all habitats for the protein count and within one habitat for the relative abundance is not significantly different according to the HSD test ($P < 0.05$).

Table 2
Number of measured proteins and proteins annotated by both a non-plant taxonomy with a bitscore lower than 135 and a function with an e-value below e^{-30} (each n = 3). In total, 1 g of litter and roots as well as 2 g of rhizosphere and bulk soil were used for protein extraction, but a final concentration of each 1 μg μL^{-1} extracted proteins was measured.

	Measured proteins		Annotated proteins	
	March	September	March	September
Litter	89,229 ± 7,095	74,228 ± 7,616	30,673 ± 611	28,960 ± 2,275
Roots	39,375 ± 23,681	157,293 ± 14,633	4,657 ± 2,876	20,138 ± 10,491
Rhizosphere	6,953 ± 1,725	8,340 ± 310	1,509 ± 324	1,629 ± 271
Soil	28,002 ± 23,388	16,280 ± 2,683	8,339 ± 8,979	4,014 ± 1,079

biosynthesis of other secondary metabolites, carbohydrate metabolism, cellular community - prokaryotes and xenobiotic biodegradation and metabolism. In bulk soil, proteins related to cellular community - prokaryotes, xenobiotic biodegradation and metabolism, cell motility and membrane transport were highly abundant. Proteins involved in amino acid metabolism, cell growth, and death, cellular community - eukaryotes, folding, sorting, and degradation, nucleotide metabolism, transcription, transport and catabolism, and xenobiotic biodegradation and metabolism were abundant in litter. On level 3, proteins involved in C fixation in photosynthetic organisms, glycolysis/gluconeogenesis, necroptosis, oxidative phosphorylation, and photosynthesis were abundant in roots. Proteins involved in citrate cycle, fructose and mannose metabolism, glycolysis/gluconeogenesis and quorum sensing were abundant in rhizosphere soil. Bulk soil showed high numbers of proteins related to ABC transporters, bacterial chemotaxis, flagellar assembly, fructose and mannose metabolism, quorum sensing and two-component system. Lastly, proteins involved in apoptosis, glycolysis/gluconeogenesis, glyoxylate and dicarboxylate metabolism, hippo signaling pathway, phagosome, purine metabolism, and pyrimidine metabolism were abundant in litter.

3.4. *Proteins involved in C and N cycling*

Most proteins assigned to CAZymes, involved in the utilization of complex C compounds, were assigned to Eukaryotes, with high

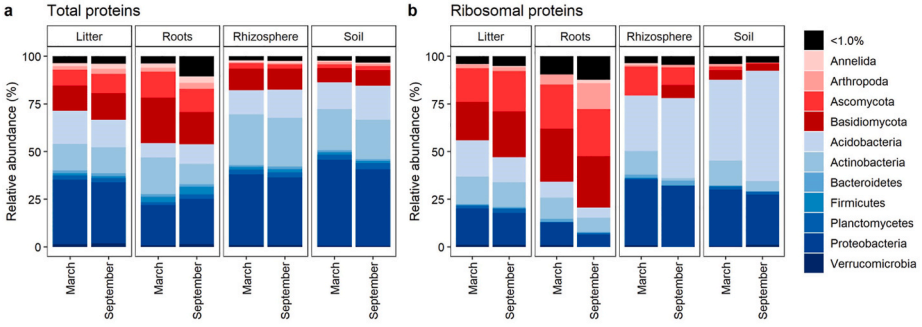

Fig. 2. The relative abundance of proteins of the most abundant phyla (excluding plants) in the forest topsoil in the total and the ribosomal protein pool. Eukaryotic phyla are shown in shades of red and bacteria in shades of blue. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

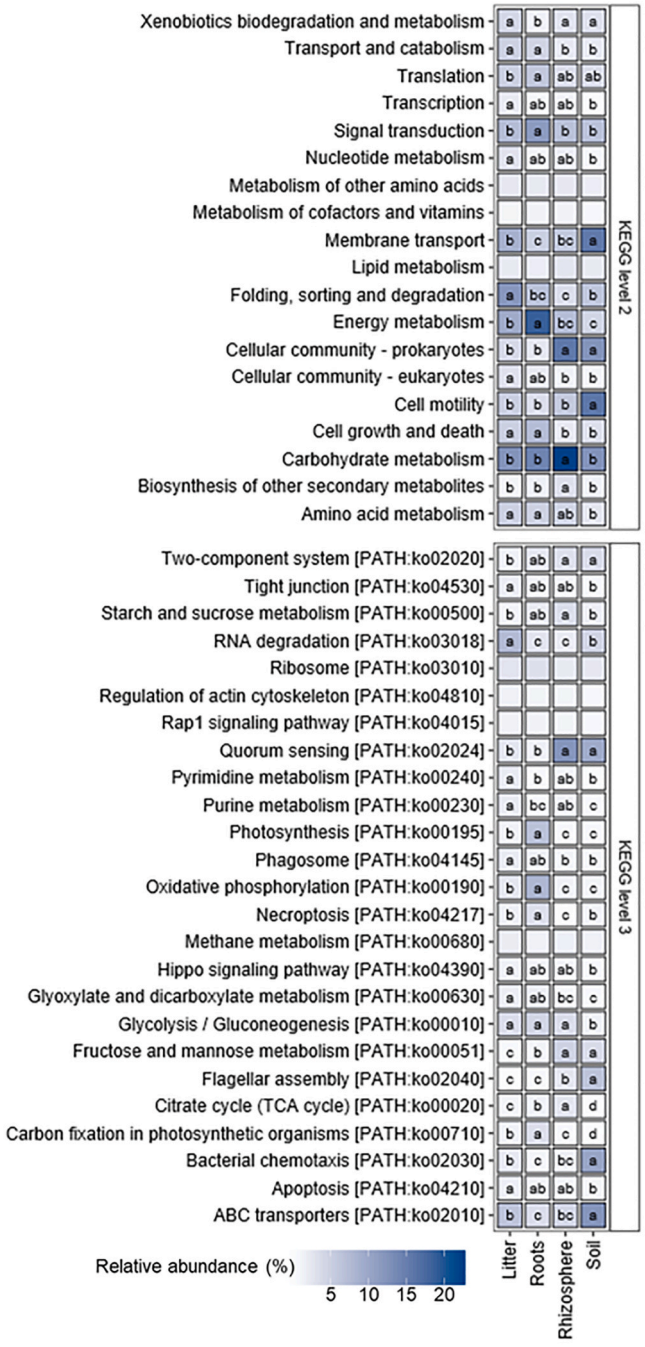

Fig. 3. Dominant functional groups of proteins in the forest topsoil metaproteome grouped by habitats. The 19 most abundant KEGG categories for level 2 and the 24 most abundant for level 3 are shown. The categories accounted for on average 98.5% and 60.7% of all level 2 and level 3 functions, respectively. Sampling times and replicates were combined for each habitat. Data with the same letter within one row is not significantly different according to the HSD test ($P < 0.05$).

Basidiomycota abundances in bulk and rhizosphere soil as opposed to high *Ascomycota* abundances in roots and litter (Fig. 4a). The share of bacterial CAZymes ranged from approximately 15% in roots to 35% in rhizosphere soil and were dominated by the *Actinobacteria*. Different sources of C were targeted in each habitat. While the CAZymes targeting plant cell wall polysaccharides (lignin, cellulose, glucuroxylans, pectin, and galactomannans) represented as much as 68% of all CAZymes in roots and 58% in litter, their share in rhizosphere and bulk soil was only 25% and 23% (Fig. 4b). Compounds of fungal origin (chitin, glucans, and glucomannans) were most frequently targeted in rhizosphere and litter (10% and 9% of CAZymes), but due to high variation, the differences among habitats were not significant. Highest utilization of cello- and xylooligosaccharides was observed in soil where they were targeted by 46% of CAZymes while their importance was low in the roots (15%). Both rhizosphere and soil were typical by a high share of CAZymes utilizing reserve compounds (starch, glycogen, and trehalose) that represented 30% and 24% of all CAZymes compared to only 11% in roots and 9% in litter. In roots, proteins involved in lignin degradation were more abundant in March, suggesting the utilization of roots that died during winter while pectin-targeting proteins were more abundant in September when mycorrhizal fungi colonized roots likely by dissolving their pectic middle lamellae (Fig. 4b).

The recovered proteins involved in N cycling were mostly of bacterial origin, with a share between 75% in roots to 95% in rhizosphere soil (Fig. 5a). Particularly the proteins of *Proteobacteria* were highly abundant in all habitats. *Acidobacteria* showed higher relative abundance in litter and in roots (especially in September), while the proteins produced by *Actinobacteria* were abundant in roots, bulk soil, and litter. Eukaryotic proteins were mostly assigned to fungi, both *Basidiomycota* and *Ascomycota*. Functionally, spruce roots and litter were dominated by proteins involved in ammonia assimilation into organic molecules whereas proteins involved in nitrification were most common in the rhizosphere soil and bulk soil, the latter showing higher share of ammonia assimilation (Fig. 5b).

4. Discussion

4.1. Remarks to the metaproteomic approach

Generally, metaproteomic approaches yield thousands of proteins per sample (Kleiner, 2019). However, with the relative CAZymes abundance of up to 5% (Žifčáková et al., 2017) only tens to hundreds of proteins identified as CAZymes will be found per sample. This is the reason why protein abundances for CAZymes have not been reported until now. The total of 139,127 proteins recovered from four forest habitats in our study make it possible to generate sufficient absolute numbers of CAZymes proteins. Indeed, the relative abundance of CAZymes proteins depended on the habitat with $1.4 \pm 0.4\%$ in litter, $3.0 \pm 0.1\%$ in roots, $0.1 \pm 0.0\%$ in rhizosphere, and $0.2 \pm 0.0\%$ in soil. Noteworthy, the extraction of proteins from the four habitats must be taken into careful consideration as not only the total amount of proteins will differ in each habitat due to the relative importance of organisms from different domains reflected in different C and N content but also the sorption will change between the habitats as their matrices comprise of different molecules (Oonk et al., 2012). In fact, no pattern between the number of measured proteins and the C/N-ratios were visible between litter, roots, and soil, which indicated that the protein extractability is based on qualitative such as different C and N sources or different sorption properties in the environment rather than quantitative measures such as the total content of C and N in the environment (Table 1). Proteins related to the N cycling showed even lower relative abundances that would make it difficult to detect with smaller protein recovery, consistent with a previous study that showed only a few proteins related to N cycling in soil (Starke et al., 2016). Admittedly, the high number of identified proteins may derive from less stringent search parameters (20 ppm precursor mass tolerance and 5% FDR). Using a stricter search parameter of 1% FDR reduced the number of peptide spectral matches from 1,401,092 (5% FDR) to 1,044,506 and the measured proteins dropped to 74,841. The FDR concept for protein identification was originally established for pure culture proteomics (Elias and Gygi, 2007), allowing for the comparison between different mass spectrometers and database search algorithms using a defined threshold of 1% (Barnouin, 2011). However, searches against large databases, such as the database used in this study, not only require long computation times

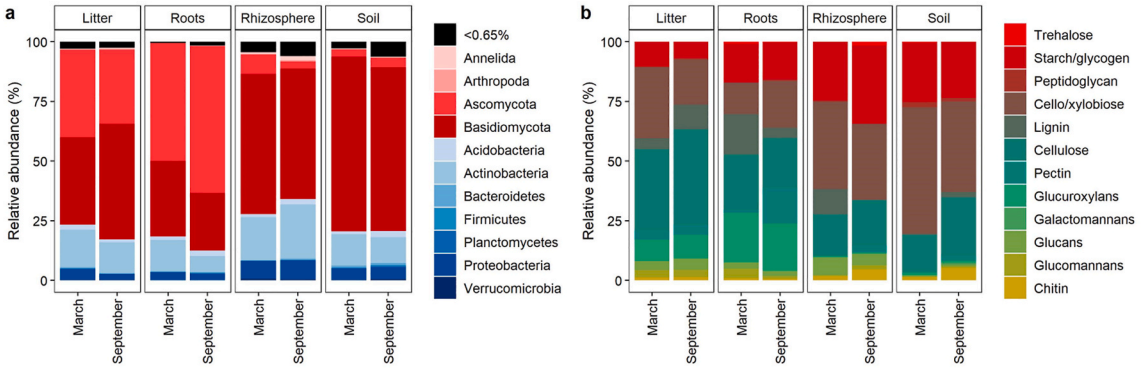

Fig. 4. The relative abundance of CAZymes of the most abundant phyla (excluding plants) in the forest topsoil (a), and the relative share of CAZymes assigned to target molecules (b). Eukaryotic phyla are shown in shades of red and bacteria in shades of blue. Chitin, glucomannans and glucans represent compounds of fungal origin, while galactomannans, glucuroxylans, pectin, cellulose lignin, cellobiose and xylobiose are components of plant cell walls or their degradation products and peptidoglycan is a component of bacterial cell wall. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

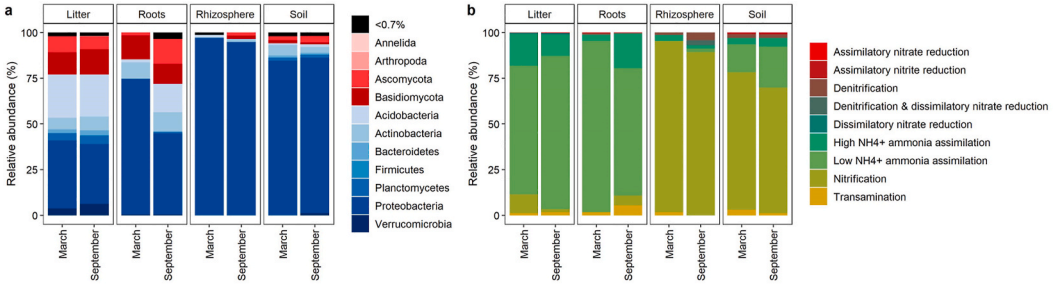

Fig. 5. The relative abundance of proteins involved in nitrogen cycling of the most abundant phyla (excluding plants) in the forest topsoil (a), and their functional distribution (b). Eukaryotic phyla are shown in shades of red and bacteria in shades of blue. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

but also decrease the number of identified proteins due to the overestimation of the FDR (Heyer et al., 2017). In fact, the limitation of the target-decoy controlled FDR approach in combination with large sequence databases in metaproteomics was responsible for missing valuable protein identifications (Muth et al., 2015) which makes FDR's higher than 1% common in metaproteomic approaches (Keiblinger et al., 2012; Schneider et al., 2012; Bastida et al., 2014; Figarska et al., 2018). That is why we believe that 5% FDR was a reasonable comprise for our study, but we also acknowledge that more stringent filtering on the probability scoring value does not only change the quantitative results but may also affect the qualitative results. Only 75.1% of the proteins were annotated by a taxonomy with high certainty, presumably due to the low number of fully sequenced and annotated genomes as they are mostly limited to those fungal and bacterial species that have undergone isolation and extensive characterization. Problematically, the vast majority of organisms were not yet studied (Pham and Kim, 2012; Martiny, 2019) and the annotation is based on the similarity to the genomes of the very few studied model organisms. However, the combination of metagenomes, metatranscriptomes and genomes of local microorganisms provided a comprehensive database that included fungal proteins, which are normally almost absent in metaproteomic data. Lastly, due to sequence homology between proteins, the annotation of deeper taxonomic depths than order level is unreliable (Starke et al., 2018) which is why a trait-based functional classification of fungi was impossible as the guilds require at least species-level identification.

4.2. *The microbial community composition of the total and the ribosomal proteins*

Only the ribosomal proteins showed a much larger contribution of fungal proteins in spruce roots and abundances like the ones of bacteria in litter. The relative contribution of fungal proteins increased with increasing C/N-ratio of the surrounding environment, consistent with higher C/N-ratios reported for fungi as compared to bacteria (Paul and Clark, 1988; Harris et al., 1997). Ribosomal proteins are components of the translation apparatus of microbes and could therefore reflect their abundance or a combination of abundance and activity, considering that more active taxa likely contain more ribosomes (Baldrian et al., 2012). Due to the fact that the ribosomal proteome and the proteome showed similar patterns in Mediterranean semi-arid soils (Bastida et al., 2016), we exclude a differential production of ribosomes as compared to all proteins between bacteria and eukaryotes. The ribosomal proteome related better to the fungal dominance of transcripts found in the very same (Žifčáková et al., 2016) or similar ecosystems of coniferous forest soils (Bailly et al., 2007; Damon et al., 2012; Takasaki et al., 2013), which is in line with the role of fungi as the key players in the plant-root-microbiome. They not only regulate plant productivity and acquire limiting nutrients (Van Der Heijden et al., 2008), but also decompose recalcitrant organic polymers in the litter (De Boer et al., 2005; Štursová et al., 2012). Noteworthy, we report similar activities between bacteria and fungi in litter as opposed to the dominance of fungi in the more decomposed litter of coniferous forest soils reported before (Ekblad et al., 2013). Among the eukaryotes, *Annelida* were also frequently found in the ribosomal proteins, highlighting the potential impact of larger eukaryotes that are generally disregarded in microbiome research. In fact, the role of microbes as predators or prey in the food-chain is generally neglected (Hall and Meyer, 1998; Haubert et al., 2009; Halvorson et al., 2016; Anderson et al., 2017), even though terrestrial omnivores and carnivores were reported to contain proteins and lipids with microbial fingerprints before (Larsen et al., 2009; Arthur

et al., 2014; Dharampal and Findlay, 2016; Ohkouchi et al., 2017; Steffan et al., 2017). The large biomass combined with relatively low C/N-ratios (Swift and Boddy, 1984) of saprotrophic cord-forming basidiomycetes (Boddy and Watkinson, 2008) make this nutrient source attractive for these soil invertebrates (Pollierer et al., 2009). Here, we provide further evidence for the involvement of these animals in the plant-root-microbiome. However, the absolute protein numbers were not sufficient to allow for a specific functional assessment of topsoil invertebrates.

4.3. The general and the specific functionality of the microbial community

Most of the most abundant level 2 (84.2%) and level 3 KEGG functions (84.6%) had significant ($P < 0.05$) differences between the habitats, indicating a different functional profile of the microbial community depending on the location in the temperate coniferous forest. Not surprisingly, the functions involved in bacterial motility, namely flagellar assembly and chemotaxis were significantly more represented in the proteome of soil, the habitat with the highest level of heterogeneity and scarce nutrients (Baldrian, 2017). The limitations of total C sources in soil as compared to litter and roots (Table 1), and with that presumably simple C sources as well are observed in the lower share of proteins involved in glycolysis compared to the other habitats. The importance of microbial sensing in soil (and rhizosphere) is supported by the elevated level of proteins involved in Quorum sensing (QS) [PATH:ko02024] as the ability to detect and to respond to population density by gene regulation (Bassler, 1999). QS bacteria release chemical signal molecules, so-called autoinducers (AI), which increase in concentration as a function of cell density. Indeed, autoinducer-2 kinase [EC:2.7.1.189] and AI-2 transport system substrate-binding protein were among the most abundant QS proteins (more information on the abundance of specific QS proteins can be found in the supplementary information, Table S1).

Fungi dominated the C cycling in each of the four habitats regardless the sampling time mainly targeting cello/xylobiose, cellulose, glucuroxylans, lignin, pectin and starch/glycogen, which corresponds with their estimated share on transcription in the same coniferous forests (Žifčáková et al., 2017) and to the proteomic analysis of decomposing beech litter (Schneider et al., 2012). All these lines of evidence support the expected major role of saprotrophic fungi in the decomposition of recalcitrant organic polymers (De Boer et al., 2005; Štursová et al., 2012). The dominance of fungi in the utilization of complex carbohydrates such as cellulose and lignin is in line with the better adaption of fungi to decompose recalcitrant plant biopolymers (Van der Wal et al., 2013). Similar to the metatranscriptomic approach, *Acidobacteria*, *Proteobacteria*, *Bacteroidetes* and *Actinobacteria* were the most abundant bacterial producers of CAZymes (Žifčáková et al., 2017). These phylotypes are known to be common in acidic forest topsoil (Žifčáková et al., 2016) and especially the members of the *Acidobacteria* and *Bacteroidetes* seem to be potent degraders of biopolymers of plant and fungal origin in forest topsoil (Lladó et al., 2016, 2019).

Our results also indicate that there are differences in the sources of C between the habitats as the spectra of genes that encode for carbohydrate-active enzymes differ. Forest litter is rich in various recalcitrant plant biopolymers and supports the activity of those microorganisms that are able to attack them as seen from a large proportion of proteins targeting plant cell wall (Johansson, 1995; Rumpel et al., 2002; Lorenz et al., 2007; Štursová et al., 2020). These proteins were also found abundant in the roots, though the reason is less obvious. The probable explanation is that the proteins degrading plant cell walls in roots either attack dead roots or serve ectomycorrhizal symbionts to colonize root tips. The latter would be supported by the observed increase of proteins related to pectin decomposition in September (Fig. 4b), the peak of ectomycorrhizal activity (Žifčáková et al., 2016). The soil and rhizosphere habitats are rather typical by the high share of proteins involved in utilization of C containing reserve compounds (e.g., starch, glycogen, trehalose), which may indicate fluctuations in C supply in these environments including the seasonal activity of roots. The ability of microbes to store C and utilize these resources may be an adaptation to these fluctuations (Žifčáková et al., 2017).

In line with the hypothesis that different microbes are involved in C and N cycling (Starke, 2017) due to their inverse CUE/NUE relationship (Mooshammer et al., 2014), bacteria dominated the N cycling in each habitat (more information on the abundance of specific proteins for each N cycling process can be found in the supplementary information, Table S2). *Acidobacteria* showed higher abundances in litter and roots, and seem to be associated with higher abundances of proteins related to ammonia assimilation, consistent with the previously reported positive correlation of the phylotype with mineral N contents (Ruppel et al., 2007; Hayatsu et al., 2008). Indeed, acidobacterial communities were more abundant and underwent significant structural changes in girdled plots (Rasche et al., 2011) in which the uptake of available nutrients such as ammonia or nitrate by trees is prevented (Högberg et al., 2001). In rhizosphere and bulk soil, nitrification was the most abundant process indicated by hydroxylamine dehydrogenase (*hao*, K10535) and three methane/ammonia monooxygenases (*pmoA*, K10944; *pmoB*, K10945 and *pmoC*, K10946). Ammonia oxidation, the first step in nitrification, is crucial for the global N cycle (Pratscher et al., 2011) and is predominantly performed by bacteria in soil (Kowalchuk and Stephen, 2001). Consistently, bacteria dominated proteins involved in N cycling regardless the habitat. The contrast between high nitrification in bulk and rhizosphere soil and the importance of the incorporation of ammonia into organic acids in litter and roots demonstrated a divergence between the habitats. While the relatively high abundance of NH_4^+ in bulk and rhizosphere soil allows its energetic use, different C/N-ratios in litter and roots allow for the preferential incorporation into the living biomass. Consistent with the lower C/N-ratios reported for soil samples as compared to litter in this temperate coniferous forest (Žifčáková et al., 2016), our data showed a similar trend of lower C/N-ratios in soil as compared to litter and roots (Table 1).

This metaproteomic analysis of major habitats in a temperate forest topsoil demonstrated the importance of both bacterial and fungal taxa. Consistent with our first hypothesis, fungal proteins dominated the decomposition of recalcitrant biopolymers in litter while bacterial proteins were more abundant in rhizosphere and soil, consistent with higher C/N-ratios in litter and roots than in soil. A niche differentiation between bacteria and fungi in N and C cycling, respectively, was unveiled. Consistent with our second hypothesis, a higher number of proteins related to ammonium assimilation was detected in litter as opposed to proteins related to nitrification in rhizosphere and soil. The metaproteome composition suggests that each of the studied habitats has specific features with the most distinct separation between soil and rhizosphere on one side, and litter and plant roots on the other side. While the latter habitats provide an essentially unlimited amount of complex C sources, their use may be limited by N availability. Our results indicate sufficient levels of N enabling nitrification but probably lower and fluctuating availability of C compounds. Despite limitations, metaproteomics seems to be suitable for the analysis of microbiome composition and its functionality, allowing a better functional focus than metagenomics while reducing the potential limitations of metatranscriptomics due to changes in expression over time or quantitative accuracy of sequencing (Feinstein et al., 2009; Hungate et al., 2015). Admittedly, the relative importance of fungi in the eukaryotic proteins was likely driven by the fungal-based database and further studies need to confirm the results with respect to other eukaryotes such as annelids and arthropods.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

This work was supported by the Czech Science Foundation (18-25706S and 20-02022Y), by the Ministry of Education, Youth and Sports of the Czech Republic (LTT17022). A portion of this research was performed under the Facilities Integrating Collaborations for User Science (FICUS; #49499) program and used resources at the DOE Joint Genome Institute and the Environmental Molecular Sciences Laboratory (grid.436923.9), which are DOE Office of Science User Facilities. Both facilities are sponsored by the Office of Biological and Environmental Research and operated under Contract Nos. DE-AC02-05CH11231 (JGI) and DE-AC05-76RL01830 (EMSL). RS thanks Nico Jehmlich for mentoring.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.soilbio.2021.108170.

References

Anderson, T.R., Hessen, D.O., 1995. Carbon or nitrogen limitation in marine copepods? Journal of Plankton Research 17, 317–331.
Anderson, T.R., Pond, D.W., Mayor, D.J., 2017. The role of microbes in the nutrition of detritivorous invertebrates: a stoichiometric analysis. Frontiers in Microbiology 7, 2113.
Arthur, K.E., Kelez, S., Larsen, T., Choy, C.A., Popp, B.N., 2014. Tracing the biosynthetic source of essential amino acids in marine turtles using δ13C fingerprints. Ecology 95, 1285–1293.
Bailly, J., Fraissinet-Tachet, L., Verner, M.C., Debaud, J.C., Lemaire, M., Wésolowski-Louvel, M., Marmeisse, R., 2007. Soil eukaryotic functional diversity, a metatranscriptomic approach. The ISME Journal 1, 632–642.
Baldrian, P., 2017. Forest microbiome: diversity, complexity and dynamics. FEMS Microbiology Reviews 41, 109–131.
Baldrian, P., Kolaiřík, M., Štursová, M., Kopecký, J., Valášková, V., Větrovský, T., Žifčáková, L., Šnajdr, J., Rídl, J., Vlček, Č., Voříšková, J., 2012. Active and total microbial communities in forest soil are largely different and highly stratified during decomposition. The ISME Journal 6, 248–258.
Barnouin, K., 2011. Guidelines for experimental design and data analysis of proteomic mass spectrometry-based experiments. Amino Acids 40, 259–260.
Bassler, B.L., 1999. How bacteria talk to each other: regulation of gene expression by quorum sensing. Current Opinion in Microbiology 2, 582–587.
Bastida, F., Hernández, T., García, C., 2014. Metaproteomics of soils from semiarid environment: functional and phylogenetic information obtained with different protein extraction methods. Journal of Proteomics 101, 31–42.
Bastida, F., Torres, I.F., Moreno, J.L., Baldrian, P., Ondoño, S., Ruiz-Navarro, A., Hernández, T., Richnow, H.H., Starke, R., García, C., Jehmlich, N., 2016. The active microbial diversity drives ecosystem multifunctionality and is physiologically related to carbon availability in Mediterranean semi-arid soils. Molecular Ecology 25, 4660–4673.
Boddy, L., Watkinson, S.C., 2008. Wood decomposition, higher fungi, and their role in nutrient redistribution. Canadian Journal of Botany 73, S1377–S1383.
Bolger, A.M., Lohse, M., Usadel, B., 2014. Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics 30, 2114–2120.
Burns, R.G., DeForest, J.L., Marxsen, J., Sinsabaugh, R.L., Stromberger, M.E., Wallenstein, M.D., Weintraub, M.N., Zoppini, A., 2013. Soil enzymes in a changing environment: current knowledge and future directions. Soil Biology and Biochemistry 58, 216–234.
Callister, S.J., Fillmore, T.L., Nicora, C.D., Shaw, J.B., Purvine, S.O., Orton, D.J., White, R.A., Moore, R.J., Burnet, M.C., Nakayasu, E.S., Payne, S.H., Jansson, J.K., Paša-Tolić, L., 2018. Addressing the challenge of soil metaproteome complexity by improving metaproteome depth of coverage through two-dimensional liquid chromatography. Soil Biology and Biochemistry 125, 290–299.
Carini, P., Marsden, P.J., Leff, J.W., Morgan, E.E., Strickland, M.S., Fierer, N., 2016. Relic DNA is abundant in soil and obscures estimates of soil microbial diversity. Nature Microbiology 2, 16242.
Chen, I.M.A., Chu, K., Palaniappan, K., Pillay, M., Ratner, A., Huang, J., Huntemann, M., Varghese, N., White, J.R., Seshadri, R., Smirnova, T., Kirton, E., Jungbluth, S.P., Woyke, T., Eloe-Fadrosh, E.A., Ivanova, N.N., Kyrpides, N.C., 2019. IMG/M v.5.0: an integrated data management and comparative analysis system for microbial genomes and microbiomes. Nucleic Acids Research 47, D666–D677.
Clemmensen, K.E., Bahr, A., Ovaskainen, O., Dahlberg, A., Ekblad, A., Wallander, H., Stenlid, J., Finlay, R.D., Wardle, D.A., Lindahl, B.D., 2013. Roots and associated fungi drive long-term carbon sequestration in boreal forest. Science 339, 1615–1618.
Damon, C., Lehembre, F., Oger-Desfeux, C., Luis, P., Ranger, J., Fraissinet-Tachet, L., Marmeisse, R., 2012. Metatranscriptomics reveals the diversity of genes expressed by eukaryotes in forest soils. PloS One 7, e28967.
De Boer, W., Folman, L.B., Summerbell, R.C., Boddy, L., 2005. Living in a fungal world: impact of fungi on soil bacterial niche development. FEMS Microbiology Reviews 29, 795–811.
Dharampal, P.S., Findlay, R.H., 2016. Mercury levels in largemouth bass (Micropterus salmoides) from regulated and unregulated rivers. Chemosphere 170, 134–140.
Ekblad, A., Wallander, H., Godbold, D.L., Cruz, C., Johnson, D., Baldrian, P., Björk, R.G., Epron, D., Kieliszewska-Rokicka, B., Kjøller, R., Kraigher, H., Matzner, E., Neumann, J., Plassard, C., 2013. The production and turnover of extramarital mycelium of ectomycorrhizal fungi in forest soils: role in carbon cycling. Plant and Soil 366, 1–27.
Elias, J.E., Gygi, S.P., 2007. Target-decoy search strategy for increased confidence in large-scale protein identifications by mass spectrometry. Nature Methods 4, 207–214.
Feinstein, L.M., Woo, J.S., Blackwood, C.B., 2009. Assessment of bias associated with incomplete extraction of microbial DNA from soil. Applied and Environmental Microbiology 75, 5428–5433.
Figarska, S.M., Gustafsson, S., Sundström, J., Ärnlöv, J., Mälarstig, A., Elmståhl, S., Fall, T., Lind, L., Ingelsson, E., 2018. Associations of circulating protein levels with lipid fractions in the general population. Arteriosclerosis, Thrombosis, and Vascular Biology 38, 2505–2518 .
Fowler, D., Coyle, M., Skiba, U., Sutton, M.A., Cape, J.N., Reis, S., Sheppard, L.J., Jenkins, A., Grizzetti, B., Galloway, J.N., Vitousek, P., Leach, A., Bouwman, A.F., Butterbach-Bahl, K., Dentener, F., Stevenson, D., Amann, M., Voss, M., 2013. The global nitrogen cycle in the Twenty first century. Philosophical Transactions of the Royal Society B: Biological Sciences 368, 20130164.
Franklin, O., Näsholm, T., Högberg, P., Högberg, M.N., 2014. Forests trapped in nitrogen limitation - an ecological market perspective on ectomycorrhizal symbiosis. New Phytologist 203, 657–666.
Grigoriev, I.V., Nikitin, R., Haridas, S., Kuo, A., Ohm, R., Otillar, R., Riley, R., Salamov, A., Zhao, X., Korzeniewski, F., Smirnova, T., Nordberg, H., Dubchak, I., Shabalov, I., 2014. MycoCosm portal: gearing up for 1000 fungal genomes. Nucleic Acids Research 42, D699–D704.
Hall, R.O., Meyer, J.L., 1998. The trophic significance of bacteria in a detritus-based stream food web. Ecology 79, 1995–2012.
Halvorson, H.M., White, G., Scott, J.T., Evans-White, M.A., 2016. Dietary and taxonomic controls on incorporation of microbial carbon and phosphorus by detritivorous caddisflies. Oecologia 180, 567–579.
Hansson, K., Fröberg, M., Helmisaari, H.S., Kleja, D.B., Olsson, B.A., Olsson, M., Persson, T., 2013. Carbon and nitrogen pools and fluxes above and below ground in spruce, pine and birch stands in southern Sweden. Forest Ecology and Management 309, 28–35.
Harris, D., Voroney, R.P., Paul, E.A., 1997. Measurement of microbial biomass N:C by chloroform fumigation-incubation. Canadian Journal of Soil Science 77, 507–514.
Haubert, D., Birkhofer, K., Fließbach, A., Gehre, M., Scheu, S., Ruess, L., 2009. Trophic structure and major trophic links in conventional versus organic farming systems as indicated by carbon stable isotope ratios of fatty acids. Oikos 118, 1579–1589.
Hayatsu, M., Tago, K., Saito, M., 2008. Various players in the nitrogen cycle: diversity and functions of the microorganisms involved in nitrification and denitrification. Soil Science & Plant Nutrition 54, 33–45.
Hettich, R.L., Pan, C., Chourey, K., Giannone, R.J., 2013. Metaproteomics: harnessing the power of high performance mass spectrometry to identify the suite of proteins that control metabolic activities in microbial communities. Analytical Chemistry 85, 4203–4214.
Heyer, R., Schallert, K., Zoun, R., Becher, B., Saake, G., Benndorf, D., 2017. Challenges and perspectives of metaproteomic data analysis. Journal of Biotechnology 261, 24–36.
Hobbie, S.E., Oleksyn, J., Eissenstat, D.M., Reich, P.B., 2010. Fine root decomposition rates do not mirror those of leaf litter among temperate tree species. Oecologia 162, 505–513.
Högberg, P., Nordgren, A., Buchmann, N., Taylor, A.F.S., Ekblad, A., Högberg, M.N., Nyberg, G., Ottosson-Löfvenius, M., Read, D.J., 2001. Large-scale forest girdling shows that current photosynthesis drives soil respiration. Nature 411, 789–792.
Huang, L., Zhang, H., Wu, P., Entwistle, S., Li, X., Yohe, T., Yi, H., Yang, Z., Yin, Y., 2018. DbCAN-seq: a database of carbohydrate-active enzyme (CAZyme) sequence and annotation. Nucleic Acids Research 46, D516–D521.
Hungate, B.A., Mau, R.L., Schwartz, E., Gregory Caporaso, J., Dijkstra, P., van Gestel, N., Koch, B.J., Liu, C.M., McHugh, T.A., Marks, J.C., Morrissey, E.M., Price, L.B., 2015. Quantitative microbial ecology through stable isotope probing. Applied and Environmental Microbiology 81, 7570–7581.
Ishiwatari, R., Uzaki, M., 1987. Diagenetic changes of lignin compounds in a more than 0.6 million-year-old lacustrine sediment (Lake Biwa, Japan). Geochimica et Cosmochimica Acta 51, 321–328.
Johansson, M.B., 1995. The chemical composition of needle and leaf litter from Scots pine, Norway spruce and white birch in scandinavian forests. Forestry 68, 49–62.
Kanehisa, M., Sato, Y., Kawashima, M., Furumichi, M., Tanabe, M., 2016a. KEGG as a reference resource for gene and protein annotation. Nucleic Acids Research 44, D457–D462.
Kanehisa, M., Sato, Y., Morishima, K., 2016b. BlastKOALA and GhostKOALA: KEGG tools for functional characterization of genome and metagenome sequences. Journal of Molecular Biology 428, 726–731.
Keiblinger, K.M., Wilhartitz, I.C., Schneider, T., Roschitzki, B., Schmid, E., Eberl, L., Riedel, K., Zechmeister-Boltenstern, S., 2012. Soil metaproteomics - comparative evaluation of protein extraction protocols. Soil Biology and Biochemistry 54, 14–24.
Kelly, R.T., Page, J.S., Luo, Q., Moore, R.J., Orton, D.J., Tang, K., Smith, R.D., 2006. Chemically etched open tubular and monolithic emitters for nanoelectrospray ionization mass spectrometry. Analytical Chemistry 78, 7796–7801.
Kim, S., Gupta, N., Pevzner, P.A., 2008. Spectral probabilities and generating functions of tandem mass spectra: a strike against decoy databases. Journal of Proteome Research 7, 3354–3363.

Kleiner, M., 2019. Metaproteomics: much more than measuring gene expression in microbial communities. MSystems 4, e00115–19.
Kowalchuk, G.A., Stephen, J.R., 2001. Ammonia-oxidizing bacteria: a model for molecular microbial ecology. Annual Review of Microbiology 55, 485–529.
Larsen, T., Lee Taylor, D., Leigh, M.B., O'Brien, D.M., 2009. Stable isotope fingerprinting: a novel method for identifying plant, fungal, or bacterial origins of amino acids. Ecology 90, 3526–3535.
Levasseur, A., Drula, E., Lombard, V., Coutinho, P.M., Henrissat, B., 2013. Expansion of the enzymatic repertoire of the CAZy database to integrate auxiliary redox enzymes. Biotechnology for Biofuels 6, 41.
Li, D., Liu, C.M., Luo, R., Sadakane, K., Lam, T.W., 2015. MEGAHIT: an ultra-fast single-node solution for large and complex metagenomics assembly via succinct de Bruijn graph. Bioinformatics 31, 1674–1676.
Lindahl, B.D., Ihrmark, K., Boberg, J., Trumbore, S.E., Högberg, P., Stenlid, J., Finlay, R. D., 2007. Spatial separation of litter decomposition and mycorrhizal nitrogen uptake in a boreal forest. New Phytologist 173, 611–620.
Liu, Y., Aebersold, R., 2016. The interdependence of transcript and protein abundance: new data–new complexities. Molecular Systems Biology 12, 856.
Lladó, S., López-Mondéjar, R., Baldrian, P., 2017. Forest soil bacteria: diversity, involvement in ecosystem processes, and response to global change. Microbiology and Molecular Biology Reviews 81, e00063–16.
Lladó, S., Větrovský, T., Baldrian, P., 2019. Tracking of the activity of individual bacteria in temperate forest soils shows guild-specific responses to seasonality. Soil Biology and Biochemistry 135, 275–282.
Lladó, S., Žifčáková, L., Větrovský, T., Eichlerová, I., Baldrian, P., 2016. Functional screening of abundant bacteria from acidic forest soil indicates the metabolic potential of Acidobacteria subdivision 1 for polysaccharide decomposition. Biology and Fertility of Soils 52, 251–260.
Lombard, V., Golaconda Ramulu, H., Drula, E., Coutinho, P.M., Henrissat, B., 2014. The carbohydrate-active enzymes database (CAZy) in 2013. Nucleic Acids Research 42, D490–D495.
López-Mondéjar, R., Brabcová, V., Štursová, M., Davidová, A., Jansa, J., Cajthaml, T., Baldrian, P., 2018. Decomposer food web in a deciduous forest shows high share of generalist microorganisms and importance of microbial biomass recycling. The ISME Journal 12, 1768–1778.
López-Mondéjar, R., Zühlke, D., Větrovský, T., Becher, D., Riedel, K., Baldrian, P., 2016. Decoding the complete arsenal for cellulose and hemicellulose deconstruction in the highly efficient cellulose decomposer Paenibacillus O199. Biotechnology for Biofuels 9, 104.
Lorenz, K., Lal, R., Preston, C.M., Nierop, K.G.J., 2007. Strengthening the soil organic carbon pool by increasing contributions from recalcitrant aliphatic bio(macro) molecules. Geoderma 142, 1–10.
Martiny, A.C., 2019. High proportions of bacteria are culturable across major biomes. The ISME Journal 13, 2125–2128.
Mayampurath, A.M., Jaitly, N., Purvine, S.O., Monroe, M.E., Auberry, K.J., Adkins, J.N., Smith, R.D., 2008. DeconMSn: a software tool for accurate parent ion monoisotopic mass determination for tandem mass spectra. Bioinformatics 24, 1021–1023.
Mooshammer, M., Wanek, W., Hämmerle, I., Fuchslueger, L., Hofhansl, F., Knoltsch, A., Schnecker, J., Takriti, M., Watzka, M., Wild, B., Keiblinger, K.M., Zechmeister-Boltenstern, S., Richter, A., 2014. Adjustment of microbial nitrogen use efficiency to carbon:Nitrogen imbalances regulates soil nitrogen cycling. Nature Communications 5, 3694.
Morrison, S., Sosnoff, J.J., Heffernan, K.S., Jae, S.Y., Fernhall, B., 2013. Aging, hypertension and physiological tremor: the contribution of the cardioballistic impulse to tremorgenesis in older adults. Journal of the Neurological Sciences 326, 68–74.
Müller, P.J., 1977. C N ratios in Pacific deep-sea sediments: effect of inorganic ammonium and organic nitrogen compounds sorbed by clays. Geochimica et Cosmochimica Acta 41, 765–776.
Muth, T., Kolmeder, C.A., Salojärvi, J., Keskitalo, S., Varjosalo, M., Verdam, F.J., Rensen, S.S., Reichl, U., de Vos, W.M., Rapp, E., Martens, L., 2015. Navigating through metaproteomics data: a logbook of database searching. Proteomics 15, 3439–3453.
Nakayasu, E.S., Nicora, C.D., Sims, A.C., Burnum-Johnson, K.E., Kim, Y.-M., Kyle, J.E., Matzke, M.M., Shukla, A.K., Chu, R.K., Schepmoes, A.A., Jacobs, J.M., Baric, R.S., Webb-Robertson, B.-J., Smith, R.D., Metz, T.O., 2016. MPLEx: a robust and universal protocol for single-sample integrative proteomic, metabolomic, and lipidomic analyses. MSystems 1, e00043–16.
Nayfach, S., Roux, S., Seshadri, R., Udwary, D., Varghese, N., Schulz, F., Wu, D., Paez-Espino, D., Chen, I.M., Huntemann, M., Palaniappan, K., Ladau, J., Mukherjee, S., Reddy, T.B.K., Nielsen, T., Kirton, E., Faria, J.P., Edirisinghe, J.N., Henry, C.S., Jungbluth, S.P., Chivian, D., Dehal, P., Wood-Charlson, E.M., Arkin, A.P., Tringe, S. G., Visel, A., Woyke, T., Mouncey, N.J., Ivanova, N.N., Kyrpides, N.C., Eloe-Fadrosh, E.A., et al., 2020. A genomic catalog of Earth's microbiomes. Nature Biotechnology in press. https://doi.org/10.1038/s41587-020-0718-6. In press.
Ohkouchi, N., Chikaraishi, Y., Close, H.G., Fry, B., Larsen, T., Madigan, D.J., McCarthy, M.D., McMahon, K.W., Nagata, T., Naito, Y.I., Ogawa, N.O., Popp, B.N., Steffan, S., Takano, Y., Tayasu, I., Wyatt, A.S.J., Yamaguchi, Y.T., Yokoyama, Y., 2017. Advances in the application of amino acid nitrogen isotopic analysis in ecological and biogeochemical studies. Organic Geochemistry 113, 150–174.
Oonk, S., Cappellini, E., Collins, M.J., 2012. Soil proteomics: an assessment of its potential for archaeological site interpretation. Organic Geochemistry 50, 57–67.
Pan, Y., Birdsey, R.A., Fang, J., Houghton, R., Kauppi, P.E., Kurz, W.A., Phillips, O.L., Shvidenko, A., Lewis, S.L., Canadell, J.G., Ciais, P., Jackson, R.B., Pacala, S.W., McGuire, A.D., Piao, S., Rautiainen, A., Sitch, S., Hayes, D., 2011. A large and persistent carbon sink in the world's forests. Science 333, 988–993.
Papp, K., Mau, R.L., Hayer, M., Koch, B.J., Hungate, B.A., Schwartz, E., 2018. Quantitative stable isotope probing with H218O reveals that most bacterial taxa in soil synthesize new ribosomal RNA. The ISME Journal 12, 3043–3045.
Paul, E.A., Clark, F.E., 1988. Soil microbiology and biochemistry. Soil Microbiology and Biochemistry, Academic Press, San Diego.
Pham, V.H.T., Kim, J., 2012. Cultivation of unculturable soil bacteria. Trends in Biotechnology 30, 475–484.
Pilegaard, K., 2013. Processes regulating nitric oxide emissions from soils. Philosophical Transactions of the Royal Society B: Biological Sciences 368, 20130126.
Pollierer, M.M., Langel, R., Scheu, S., Maraun, M., 2009. Compartmentalization of the soil animal food web as indicated by dual analysis of stable isotope ratios (15N/14N and 13C/12C). Soil Biology and Biochemistry 41, 1221–1226.
Prahl, F.G., Ertel, J.R., Goni, M.A., Sparrow, M.A., Eversmeyer, B., 1994. Terrestrial organic carbon contributions to sediments on the Washington margin. Geochimica et Cosmochimica Acta 58, 3035–3048.
Pratscher, J., Dumont, M.G., Conrad, R., 2011. Ammonia oxidation coupled to CO2 fixation by archaea and bacteria in an agricultural soil. Proceedings of the National Academy of Sciences of the United States of America 108, 4170–4175.
Rasche, F., Knapp, D., Kaiser, C., Koranda, M., Kitzler, B., Zechmeister-Boltenstern, S., Richter, A., Sessitsch, A., 2011. Seasonality and resource availability control bacterial and archaeal communities in soils of a temperate beech forest. The ISME Journal 5, 389–402.
Rumpel, C., Kögel-Knabner, I., Bruhn, F., 2002. Vertical distribution, age, and chemical composition of organic carbon in two forest soils of different pedogenesis. Organic Geochemistry 33, 1131–1142.
Ruppel, S., Torsvik, V., Daae, F.L., Øvreås, L., Rühlmann, J., 2007. Nitrogen Availability Decreases Prokaryotic Diversity in Sandy Soils. Biology and Fertility of Soils. Springer.
Schneider, T., Keiblinger, K.M., Schmid, E., Sterflinger-Gleixner, K., Ellersdorfer, G., Roschitzki, B., Richter, A., Eberl, L., Zechmeister-Boltenstern, S., Riedel, K., 2012. Who is who in litter decomposition Metaproteomics reveals major microbial players and their biogeochemical functions. The ISME Journal 6, 1749–1762.
Simon, J., Klotz, M.G., 2013. Diversity and evolution of bioenergetic systems involved in microbial nitrogen compound transformations. Biochimica et Biophysica Acta (BBA) - Bioenergetics 1827, 114–135.
Sparacino-Watkins, C., Stolz, J.F., Basu, P., 2014. Nitrate and periplasmic nitrate reductases. Chemical Society Reviews 43, 676–706.
Starke, R., 2017. Untersuchung der Energie-und Nährstoffflüsse mikrobieller Gemeinschaften. University of Hohenheim.
Starke, R., Jehmlich, N., Bastida, F., 2018. Using proteins to study how microbes contribute to soil ecosystem services: the current state and future perspectives of soil metaproteomics. Journal of Proteomics 198, 50–58.
Starke, R., Kermer, R., Ullmann-Zeunert, L., Baldwin, I.T., Seifert, J., Bastida, F., von Bergen, M., Jehmlich, N., 2016. Bacteria dominate the short-term assimilation of plant-derived N in soil. Soil Biology and Biochemistry 96, 30–38.
Steffan, S.A., Chikaraishi, Y., Dharampal, P.S., Pauli, J.N., Guédot, C., Ohkouchi, N., 2017. Unpacking brown food-webs: animal trophic identity reflects rampant microbivory. Ecology and Evolution 7, 3532–3541.
Sterner, R.W., Elser, J.J., 2002. Ecological stoichiometry: the biology of elements. In: Molecules to the Biosphere. Princeton University Press.
Štursová, M., Šnajdr, J., Koukol, O., Tláskal, V., Cajthaml, T., Baldrian, P., 2020. Long-term decomposition of litter in the montane forest and the definition of fungal traits in the successional space. Fungal Ecology 46, 100913.
Štursová, M., Žifčáková, L., Leigh, M.B., Burgess, R., Baldrian, P., 2012. Cellulose utilization in forest litter and soil: identification of bacterial and fungal decomposers. FEMS Microbiology Ecology 80, 735–746.
Sutton, M.A., Reis, S., Riddick, S.N., Dragosits, U., Nemitz, E., Theobald, M.R., Tang, Y.S., Braban, C.F., Vieno, M., Dore, A.J., Mitchell, R.F., Wanless, S., Daunt, F., Fowler, D., Blackall, T.D., Milford, C., Flechard, C.R., Loubet, B., Massad, R., Cellier, P., Personne, E., Coheur, P.F., Clarisse, L., Van Damme, M., Ngadi, Y., Clerbaux, C., Skjøth, C.A., Geels, C., Hertel, O., Kruit, R.J.W., Pinder, R.W., Bash, J.O., Walker, J. T., Simpson, D., Horváth, L., Misselbrook, T.H., Bleeker, A., Dentener, F., de Vries, W., 2013. Towards a climate-dependent paradigm of ammonia emission and deposition. Philosophical Transactions of the Royal Society B: Biological Sciences 368, 20130166.
Swift, M.J., Boddy, L., 1984. Animal–microbial interactions in wood decomposition. In: Anderson, J.M., Rayner, A.D.M., Walton, D.W.H. (Eds.), Invertebrate–Microbial Interactions. Cambridge University Press, Cambridge, pp. 89–131.
Takasaki, K., Miura, T., Kanno, M., Tamaki, H., Hanada, S., Kamagata, Y., Kimura, N., 2013. Discovery of glycoside hydrolase enzymes in an avicel-adapted forest soil fungal community by a metatranscriptomic approach. PloS One 8, e55485.
Tukey, J.W., 1949. Comparing individual means in the analysis of variance. Biometrics 5, 99–114.
Urabe, J., Watanabe, Y., 1992. Possibility of N or P limitation for planktonic cladocerans: an experimental test. Limnology & Oceanography 37, 244–251.
Van Der Heijden, M.G.A., Bardgett, R.D., Van Straalen, N.M., 2008. The unseen majority: soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. Ecology Letters 11, 296–310.
Van der Wal, A., Geydan, T.D., Kuyper, T.W., De Boer, W., 2013. A thready affair: linking Fungal diversity and community dynamics to terrestrial decomposition processes. FEMS Microbiology Reviews 37, 477–494.
Vitousek, P.M., Menge, D.N.L., Reed, S.C., Cleveland, C.C., 2013. Biological nitrogen fixation: rates, patterns and ecological controls in terrestrial ecosystems. Philosophical Transactions of the Royal Society B: Biological Sciences 368. https://doi.org/10.1098/rstb.2013.0119.

Von Bergen, M., Jehmlich, N., Taubert, M., Vogt, C., Bastida, F., Herbst, F.A., Schmidt, F., Richnow, H.H., Seifert, J., 2013. Insights from quantitative metaproteomics and protein-stable isotope probing into microbial ecology. The ISME Journal 7, 1877–1885.
Wilmes, P., Heintz-Buschart, A., Bond, P.L., 2015. A decade of metaproteomics: where we stand and what the future holds. Proteomics 15, 3409–3417.
Wong, D.W.S., 2009. Structure and action mechanism of ligninolytic enzymes. Applied Biochemistry and Biotechnology 157, 174–209.
Žifčáková, L., Větrovský, T., Howe, A., Baldrian, P., 2016. Microbial activity in forest soil reflects the changes in ecosystem properties between summer and winter. Environmental Microbiology 18, 288–301.
Žifčáková, L., Větrovský, T., Lombard, V., Henrissat, B., Howe, A., Baldrian, P., 2017. Feed in summer, rest in winter: microbial carbon utilization in forest topsoil. Microbiome 5, 122.